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THE EFFECT OF T-DEPENDENT ANTIGEN ON THE PROFILES OF CIRCULATING WHITE BLOOD CELLS, CORTICOSTERONE, T_3, T_4 AND GLUCOSE DURING THE INITIATION OF HUMORAL IMMUNE RESPONSE IN WHITE LEGHORNE COCKERELS.

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ABSTRACT

This study was conducted to monitor the early physiological events during the initiation of the humoral immune response. This would eventually benefit the development of superior poultry immunization program. One hundred and twenty male white Leghorn cockerels aged eight–week–old were injected intravenously with either 0.5 ml of 10% sheep red blood cells (SRBC), a T-dependent antigen, or saline. Blood samples were collected at 0, 1, 3, 6, 9 and 24h following the injection. Total white blood cells (WBC), differential counts, corticosterone, T_3, T_4 and glucose concentrations were measured in all blood samples.

Total WBC counts, lymphocytes (L) and monocytes decreased significantly at 3, 6 and 9h post SRBC injection. However, circulating heterophils (H) and H/L ratio increased significantly at 1, 3, 6 and 9h post SRBC injection.

Serum corticosterone and glucose concentrations increased significantly at 3h and 6h and at 3 h post SRBC injection respectively. On the other hand, serum T_3 and T_4 decreased significantly at 3h post SRBC injection.

There were no significant changes in total WBC count, lymphocytes and heterophils count, serum corticosterone and glucose levels following saline injection. However, monocytes decreased significantly at 6h and 9h. T_4 decreased significantly at 3h, while T_3 increased at 1h post saline injection as compared to 0h.

These results showed that injection of the T-dependent antigen, SRBC, initiates a wide variety of hormonal, immune logical and metabolic changes in the chickens. These changes may explain the interaction between the neuroendocrine and immune system.

Keywords: Chicken, immune response, white blood cells, corticosteron, T_3, T_4, glucose.

INTRODUCTION

In recent years, the interaction of neuro-endocrine systems has become an area of considerable interest both in mammalian and avian immunology. These interactions are considered to represent a neuroendocrine- immune network that regulates differentiation, development and functions of immune cells (Marsh and Scanes, 1994; Mashaly *et al.*, 1998). The neuroendocrine system can communicate with the immune system either through the direct innervation of immune organs (Felten *et al.*, 1985; Ackerman *et al.*, 1987), or via hormones that transmit signals at a slower rate, but act systemically and for a longer period of time. The latter include: 1) Products of the hypothalamus such as gonadotropin releasing factor, corticotropin releasing factor (CRF) hormones of the pituitary such as

FSH, LH, ACTH, growth hormone and prolactin (Grossman, 1989; Kordon and Drouva, 1992; Marsh and Scanes, 1994; Mashaly *et al.,* 1998; Stanisz *et al.,* 1994). 2) Hormones from the thyroid, adrenal cortex, pineal gland and gonads (Grossman 1989; Landsman *et al.,* 2001; Brennan *et al.,* 2002). One of the most convincing indications that lymphoid cells and tissues are responsive to avariety of endocrine mediators is the expression of a range of hormonal receptors by these cells and tissues (Grossman *et al.,* 1991; Roszman and Brooks, 1988).

Another indicator for the interaction is the fact that primary lymphoid organs of avian species (i.e., the thymus and the bursa of fabricius) are also known to function as endocrine organs. These produce hormonal products of lymphoid cells that may feed back on the neuroendocrine system. McGillis *et al.,* (1985) found that thymic hormone (thymosin) elevated corticosterone levels is rodents. Bursal hormone (bursin) has also been shown to exert distinct feed back regulatory effects on the pituitary- adrenal axis (Guellati *et al.,* 1991) and on the pituitary and hypothalamus (Jankovic, 1987). Some lymphoid tissue and cells secrete a variety of cytokines like interleukin- 1 (IL-1) which stimulates the hypothalamus to produce CRF acting upon the adenohypophysis to release ACTH. This in turn stimulates the adrenal cortex to secrete corticosterone (Besodovsky *et al.,* 1986).

The present experiment was conducted to study changes in the profiles of circulating white blood cells, serum corticosterone, triiodothyronine (T_3), thyroxin (T_4) and glucose following SRBC antigen challenge in white leghorn cockerels.

MATERIALS AND METHODS

The present study was carried out at the Poultry Research Center, Animal Production Department, Faculty of Agriculture, Cairo University.

Animals:

One hundred twenty 8-week old White Leghorn cockerels were used in this study. The birds were housed in batteries, with feed and water available *ad libitum,* and exposed to 16h light and 8h dark per day.

Immunization and bleeding protocol:

At 8 weeks of age, the birds were divided into two groups. The first group was injected intravenously (via brachial) with 0.5 ml of 10% SRBC prepared in 0.9% saline. The second group was injected with sterile saline (0.9% NaCL) using the same route of injection. Ten birds from each treatment group were bled before injection. Ten additional birds from each treatment group were bled at 1, 3, 6, 9, and 24h following injection. No bird was bled more than once. To insure that handling the birds would not affect corticosterone levels (Trout *et al.,* 1988), the blood samples were drawn within one min after the birds were removed from cages. Approximately 3ml of blood were drawn from the brachial vein of each bird. About 1ml of blood was placed in heparinized tubes to be used for white blood cells counts, the remainder was allowed to clot to provide serum for hormone assays.

Separated serum was stored at –02°C until hormones were determined.

Total and differential WBC counts:

Total WBCs count were made by diluting whole blood with cresyl brilliant blue dye, then counting the leukocytes under microscope using a hemocytometer to calculate WBC/mm^3. Blood smears for differential counts, were prepared and stained with Hema-3 stain .A total of 100 WBC were counted and the percentage of heterophils and lymphocytes was determined.

Hormone assays:

Radioimmunoassay (RIA) kits (Diagnostic Los Angeles 90045-5597 measured serum corticosterone, triiodothyronine (T_3), and thyroxine (T_4).

Blood glucose:

Serum glucose values were determined using colorimetric determination method (Stanbio laboratory. Inc., procedure No. 1075).

Statistical analysis:

Data were analyzed using a two-way analysis of variance using treatment (antigen vs. saline) and time (0, 1, 3, 6, 9 and 24h) as the two main effects using the general Linear Models procedure (SAS Institute, 1988). Separation of means were done using Duncan's multiple range test. Unless otherwise specified, all significant differences reported in this paper are between the values of the treated and the control birds within time periods as well as between the treated birds at zero time and other time period. The significance level was set at ≤ .05.

RESULTS

Change in circulating white blood cells:

The effect of SRBC and saline injection on total WBC and lymphocyte percentage are shown in Figure 1 and 2. There was a significant decrease in total WBC and lymphocyte percentage at 1, 3, 6 and 9h post-SRBC injection compared to 0h or compared to the corresponding time points in the saline – injected birds.

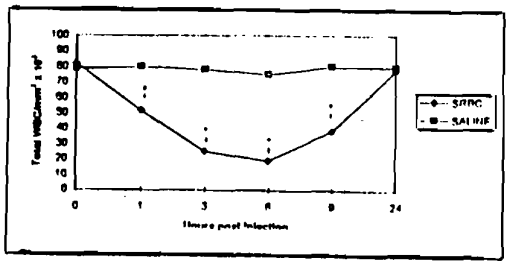

Figure 1: Changes in circulated numbers of total WBC per mm^3 in the blood of SRBC and Saline-injected Leghorn cockerels.
*** indicates significance (P≤0.05) compared to 0 h within treatments (SRBC or Saline).**
† indicates significance (P≤0.05) between treatments (SRBC or Saline) at each specific time point.

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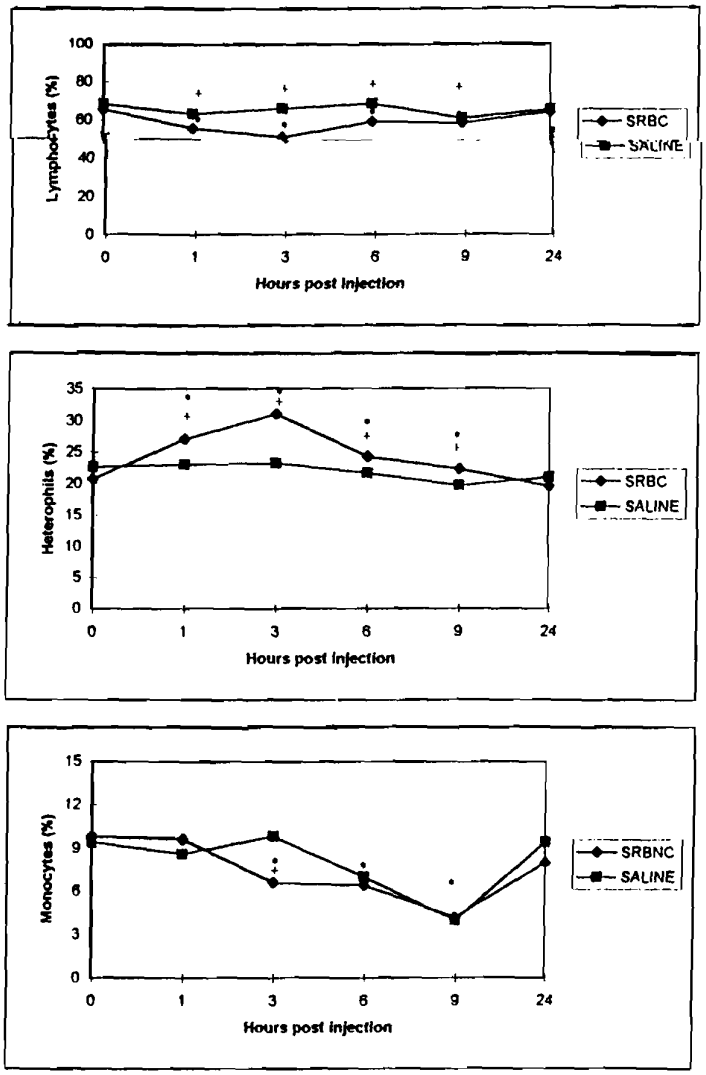

Figure 2: Changes in circulated percentages of lymphocytes, heterophils and monocytes in the blood of SRBC and Saline-injected Leghorn cockerels.
* indicates significance ($P \leq 0.05$) compared to 0 h within treatments (SRBC or Saline).
+ indicates significance ($P \leq 0.05$) between treatments (SRBC or Saline) at each specific time point.

In contrast, the heterophils percentage rose significantly at 1h, and remained elevated until 9h post-SRBC injection from its level at 0h, or compared to the corresponding time points in the saline injected birds (Figure 2).

Heterphil to lymphocyte ratio (H/L ratio) are presented in Figure 3. Following SRBC injection H/L ratio was elevated significantly at 1, 3, 6 and 9h post injection from 0h or compared to those counterparts that were injected saline.

Total WBC counts, lymphocytes, heterophils percentages and H/L ratio returned at 24h post- SRBC injection to its original values.

Circulating monocytes gradually decreased and reach its lowest count at 9h post SRBC or saline injection. In SRBC – injected birds, circulating monocytes decreased significantly at 3, 6 and 9h post injection compared to 0h. While in saline – injected birds, monocytes decreased significantly at 6 and 9h postinjection compared to 0h. The significant differences in circulating monocytes between SRBC and saline –injected birds were only at 3h post injection.(Figure 2)

Serum corticosterone :

The effect of SRBC injection on serum corticosterone levels is shown in Figure 4. Corticosterone levels rose significantly at 3h post – SRBC injection and remained elevated until 6h post- SRBC injection compared to saline group levels at the same time points. Additionally in SRBC – injected birds corticosterone levels were significantly higher at 3 and 6h than levels at 0h.

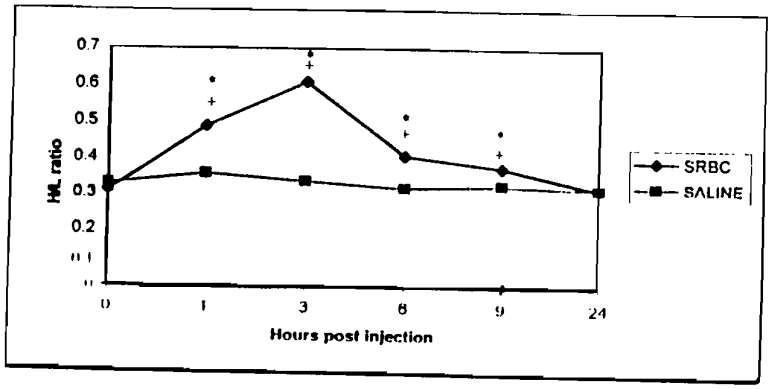

Figure 3: Changes in H/L ratio in the blood of SRBC and Saline-injected Leghorn cockerels.
* indicates significance ($P \leq 0.05$) compared to 0 h within treatments (SRBC or Saline).
+ indicates significance ($P \leq 0.05$) between treatments (SRBC or Saline) at each specific time point.

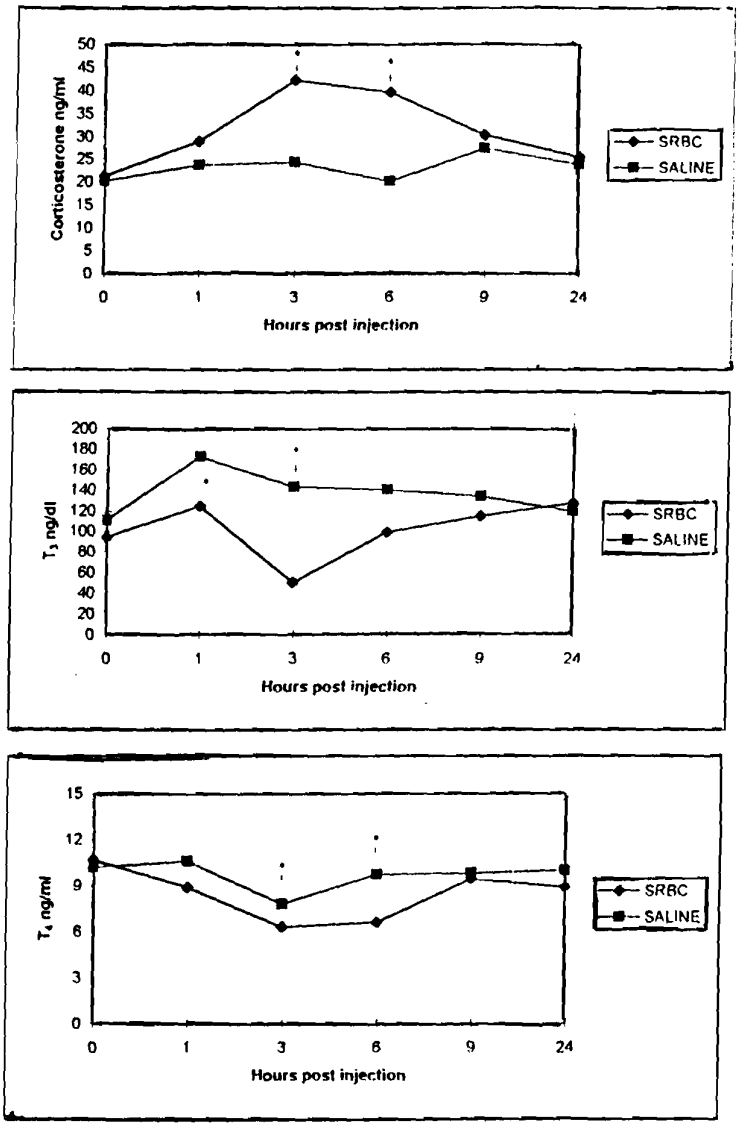

Figure 4: Changes in total serum concentrations of corticosterone, T_3 and T_4 in the blood of SRBC and Saline-injected Leghorn cockerels.
* indicates significance ($P \leq 0.05$) compared to 0 h within treatments (SRBC or Saline).
+ indicates significance ($P \leq 0.05$) between treatments (SRBC or Saline) at each specific time point.

Serum T_3 and T_4

As shown in Figure 4, T_3 levels decrease significantly at 3h post SRBC injection compared to 0h or compared to saline group levels at the same time point. On the other hand T_3 concentrations were elevated significantly at 1h post saline injection compared to 0h.

Serum T_4 levels decreased significantly at 3 and 6h post SRBC injection and at 3 post saline injection from 0h levels. Also there were significant decrease in T_4 levels at 3 and 6 h post SRBC injection compared to saline injected birds at the same time points (Figure 4).

Serum glucose:

At 6 h after SRBC injection serum glucose increased significantly compared to those injected with saline at the same time points or compared to the levels at 0h (Figure 5).

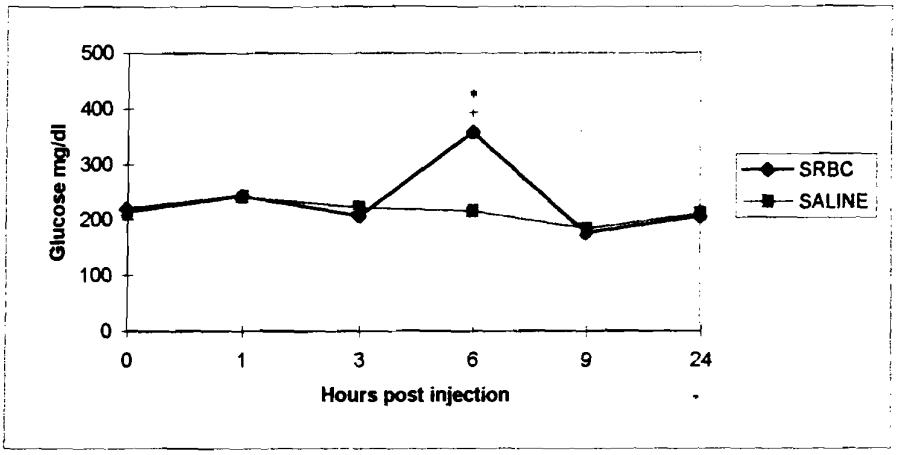

Figure 5: Changes in serum glucose in the blood of SRBC and Saline-injected Leghorn cockerels.
* indicates significance ($P \leq 0.05$) compared to 0 h within treatments (SRBC or Saline).
+ indicates significance ($P \leq 0.05$) between treatments (SRBC or Saline) at each specific time point.

DISCUSSION

The present study indicates that initiation of humoral immune response against T-dependent antigens, such as SRBC, is accompanied by an alteration in the profile of circulating leukocytes and their subtypes. Some circulating hormone levels were also altered in the early hours, following immunization. The injection of SRBC seems to activate the hypothalamic pituitary- adrenal axis (HPA-axis) and leads to a significant rise in corticosterone levels as early as 3h post injection and continued to 6h post injection. Several reports have indicated that exposure to lipopolysaccharide (LPS) (T- independent antigen) or SRBC (T- dependent antigen) results in the activation of the HPA- axis leading to glucocorticoid production (Takao *et al.*, 1997; Ottaway *et al.*, 1998; Givalois *et al.*, 1994; Al- Dokhi, 1996).

Chrousos (1995) reported that injection of some antigen like LPS stimulates the production of pro-inflammatory cytokines such as IL-1, TNF- α and IL-6 that can activate different elements of the HPA-axis leading to glucocorticoid release. Furthermore, glucocorticoid release leads to inhibition of further secretion of many cytokines, preventing an over activation of the immune system (Almawi *et al.*, 1996).

The increase in corticosterone level, in the present study, appears to decrease the number of most circulating leukocytes except heterophils. This decline could either be due to steriod lysis of cells (Claman *et al.*, 1971), programed cell death (apoptosis) (Thompson, 1999), or its distribution to secondary lymphoid organs (Mashaly *et al.*, 1998; Fauci, 1975). Leukocytes are important for the body's defense against invading microorganisms. To maintain a state of readiness, leukocytes circulate continuously through the blood and lymphatic tissue. This circulation ensures continuous surveillance that is a prerequisite for an efficient defense system. Peripheral lymphocytes and monocytes often traffic to secondary lymphoid organs where antigen presentation may take place (Tizard, 1996).

Previous researches indicated which subset of lymphocytes influenced by the concentration of corticosterone. The early study of Trout *et al.* (1988), suggested that entry of antigen into secondary lymphoid tissues, such as the spleen, lymph nodes and bone marrow, caused these tissue to trap lymphocytes in order to concentrate antibody producing cells (B-cell) with antigen. However, the same authors in 1996, found no changes in the B-cell distribution in the circulation following the injection of either bovine serum albumine (BSA) or SRBC. Berezi, (1986) also reported that circulating B-cell changed to a lesser extant than T-cells in response to corticosterone. Trout *et al.* (1996) showed that injection of the T- dependent soluble peptide antigen BSA caused a decrease in the percentages of T- helper (CD4) and T-cytotoxic (CD8) in the circulation as early as 3 to 6h post injection. The results of Agarwal *et al.* (1998) explained that glucocorticoids can shift the T-helper1/ T-helper2 cytokine balance towards a predominant T-helper2 response. Glucocorticoids can do this by suppressing the production of IL-12, the main inducer of T-helper1 cytokines such as IFN-γ and IL-2 (Dekruyff *et al.*, 1998).

The observed increase in circulating heterophils in the present study may also be related to the increase in corticosterone following SRBC injection. Exogenous corticosterone has been reported to increase circulating heterophils (Siegel, 1968; Trout *et al.*, 1988). Elevation in plasma corticosterone can apparently alter relative heterophil and lymphocyte percentages in a manner consistent with the change we observed in the leukocytes count. Following SRBC injection L and H decrease and increase, respectively, in the circulation. This resulted in a significant rise in the H/L ratio from 1 to 6 h postinjection. The greatest ratio was observed at 3h post injection which was almost twofold higher than that at 0 h. The H/L ratio has been documented as an index of stress in chickens (Gross and Siegel, 1983).

The observed decrease in the circulating monocyte, in the present study, may also be accounted for, in part, by its trapping in the secondary lymphoid organs, as macrophages. This process is needed to participate in antigen processing and presentation (Uranne and Allen, 1987). Corticosterone has also been suggested to cause the migration of the mononuclear phagocytes out of the circulation and into the secondary (Thompson and Furth, 1970). In the present study circulating monocytes decreased post saline injection without apparent reasons.

The present study showed that circulating levels of T_3 and T_4 decreased during the early hours of the immune response. Changes in T_3 levels during an immune response have been reported previously (Besodovsky *et al.*, 1975; Keast and Ayre, 1980; Trout *et al.*, 1988). Kahl *et al.* (2000) explained that injection of LPS reduces the activity of the enzyme 5\ - deiodinase that converts T_4 to T_3 in the lever. This finding may interpret our observation of a decline in T_3 concentration at 3h post SRBC injection Trout *et al.* (1988) suggested that T_3 may be associated with the differentiation of B-cells to plasma cells and the production of antibody.

The present results showed that serum glucose was also affected by SRBC injection. Increased circulating glucocorticoid levels are known to increase catabolism of protein and fatty tissue through gluconeogenesis with a resultant increase in circulating glucose (Siegel 1971; Puvadolpirod and Thaxton 2000; Zulkifli *et al.*, 2000). Bisbis *et al.* (1994) have demonstrated that exogenous corticosterone effectively induced insulin resistance in chicken.

In conclusion, the current study may explain that the initiation of humoral immune response involves the activation of HPA-axis, which resultes in an increase in serum corticosterone levels. This increase causes a decrease in total WBC counts, lymphocytes, and monocytes. However, heterophil percentages and serum glucose levels increased. The decrease in serum T_3 may be related to the increase in serum corticosterone or the reduction in converting T_4 to T_3.

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" التغير فى عدد كرات الدم البيضاء – مستوى هرمون الكورتيكوسترون ومستوى الجلوكوز فى الدم أثناء الإستجابة المناعية الأولية– T4 – T3 – الليمفاوية فى ذكور دجاج اللجهورنTضد الإنتيجينات ذات الإعتماد على خلايا الأبيض "

عبد الرحمن محمد محمد عطا

كلية الزراعة – قسم الإنتاج الحيوانى–جامعة القاهرة

أجرى هذا ابحث بغرض تقدير الأحداث الفسيولوجية المبكرة المصاحبة لبدء الاستجابة المناعية المصلية. والتى قد تفيد فى تطوير برامج التحصين للدواجن. أستخدم فى هذه الدراسة ١٢٠ كتكوت ذكر لجهورن أبيض. قسمت الطيور عند عمر ٨ أسابيع إلى مجموعتين حقنت الأولى ٠,٥ سم^{٣} أنتيجين كرات الدم الحمراء للغنم بينما حقنت المجموعة الثانية بمحاول فسيولوجى وقد تم الحقن فى وريد الجناح. أخذت عينات الدم من كلا من المجموعتين عند صفر ، ١ ،٣ ، ٦ ، ٩ و ٢٤ ساعة بعد الحقن. وتم تقدير عدد ونسب كرات الدم البيضاء– مستوى هرمون الكورتيكوسترون - $T_3 - T_4$ فضلا عن مستوى الجلوكوز.

ولوحظ الآتي : انخفاض عدد كرات الدم البيضاء ونسبة كرات الدم البيضاء الليمفاوية (L) والأحادية بعد ٣ ، ٦ و ٩ ساعات من حقن الانتيجين. بينما أرتفع نسبة كرات الدم البيضاء الحمضية (H) ونسبة L : H بصورة معنوية عند ١ ، ٣ ، ٦ و ٩ ساعة بعد حقن الأنتيجين.إرتفع مستوى هرمون الكوريتكوسترون بعد٣ ، ٦ ساعة بينما أرتفع تركيز الجلوكوز بعد ٣ ساعات من حقن الأنتيجين.

من ناحية أخرى إنخفض تركيز هرمون $T_3 - T_4$ بصورة معنوية بعد ٣ ساعات من حقن الأنتيجين. هذا ولم يلاحظ أى تغيرات معنوية فى عدد كرات الدم البيضاء ونسبة الخلايا الليمفاوية والحامضية وتركيز هرمون الكورتيكوسترون ومستوى الجلوكوز بعد حقن المحلول الفسيولوجى بينما انخفضت نسبة الخلايا الأحادية بعد ٦ ، ٩ ساعة من الحقن وإزداد تركيز T_4بعد ٣ ساعات وتركيز T_3 بعد ساعة من حقن المحلول الفسيولوجى.

هذه النتائج تشير إلى أن استخدام أنتجينات معتمدة على خلايا T الليمفاوية مثل كرات الدم الحمراء للغنم تؤدى إلى تغيرات هرمونية ومناعية وتمثيل غذائي في الدجاج. وهذه التغيرات توضح الارتباط والتفاعل بين الجهاز العصبي الهرموني بالاستجابة المناعية.